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CYMIRAFEN - A protein-conjugate cancer therapeutic that selectively targets stem cells in multiple types of cancer

Molecular targets: LGR4, LGR5 and LGR6 receptors uniquely expressed at high levels in cancer stem cells

Cellular target: LGR4, LGR5 or LGR6-positive cancer cells

Indication: gastric, esophageal, colon, endometrial, ovarian, lung, breast, and skin cancers

Cymirafen targets cancer stem cells

- **All cancers rely on** primitive **stem-like cells** for growth and metastasis
- The **LGR4, LGR5 and LGR6** family of receptors **mark active stem cells** in essentially all dividing epithelia

  Validated by >100 publications using in vitro and in vivo biologic systems

  Validated in >50 molecular and lineage tracing systems

- **LGR5 in particular is over-expressed in the stem cells** of many common types of **cancer** including colon, gastric, endometrial, ovarian, lung, breast, skin, etc)
- **Cymirafen** uses the binding domain of a **normal RSPO1 ligand** to selectively **deliver a cytotoxin (MMAE)** to LGR4, LGR5 and/or LGR6 positive stem cells.
- After binding to an LGR **cymirafen is rapidly endocytosed** and free **MMAE is released intracellularly**
- There are no approved drugs that uniquely target tumor stem cells – c**ymirafen fills this void**

Unique target and cell-validated basis of selectivity

- **Cymirafen is extremely potent**: IC_{50} values 0.5 – 10 nM in cell line panels in 2D and 3D spheroid cultures
- **Cymirafen is highly selective** for cells that express the LGRs
  - Extensive validation that cell kill is depedent on specific LGR binding as exemplified both in vitro and in vivo with LGR5 overexpressing cells
- **Efficacy in multiple human xenograft models**:
  - Documented efficacy in colorectal, gastric, ovarian cancers and neuroblastoma thus far
  - Data sufficient for IND-enabling studies
- **Favorable pharmacokinetic profile** – PK in mice and rats supports weekly dosing
- **Favorable toxicology profile in a dose-ranging rat study**
  - No serum chemistry abnormalities; adverse events limited to neutropenia/thrombocytopenia
  - $HNSDT_{10}$ in rats 75 mg/kg

Clearly defined regulatory pathway

- **Regulatory**
  - **Cymirafen** is an **ADC-like biologic** so it exploits the extensive experience with development of MMAE ADCs
- **CMC**
  - **Cymirafen** is a **robust and stable protein**
  - Purification uses standard 2-step Ni-NTA capture and ion exchange technology
  - High level production by stable CHO clones has been established
- **Intellectual property**
  - **Issued patents** in USA and EU
  - Recently filed patent applications with potential for **broad territorial coverage**

Cymirafen (FcF2-MMAE) structure

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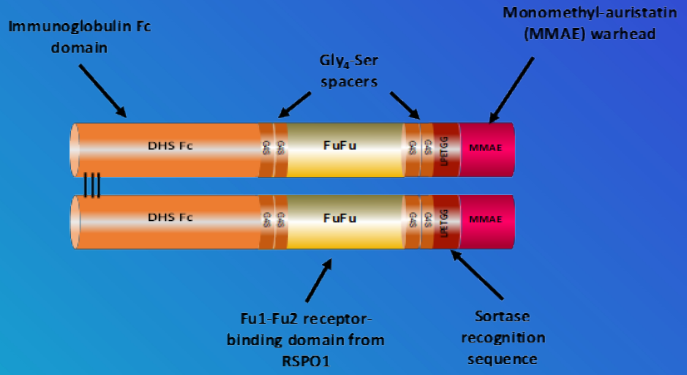
Cymirafen uses the **LGR4/LGR5/LGR6 receptor** binding domain of their natural ligand, RSPO1, to **target MMAE to cancer stem cells**

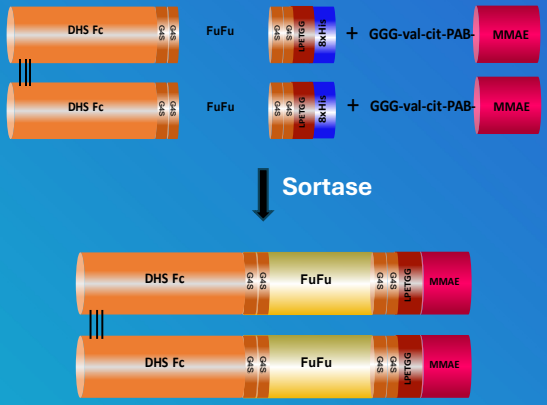
Cymirafen has 3 parts:

1. An immunoglobulin Fc domain
2. The LGR4/LGR5/LGR6 binding domain from RSPO1
3. An LPETGG tag that allows site-specific conjugation of the cytotoxin MMAE by sortase A



Novel ADC-like first-in-class protein drug conjugate

Site specific linkage of MMAE to cymirafen



MW of dimer
85.4 Da

- **Sortase reaction** adds MMAE only at the two C-terminal site and no other sites
- DAR 1.8
- Very stable peptide bond linkage
- MMAE is coupled via a val/cit-PAB-MMAE cleavable linker
- Efficiency of sortase reaction: >85%
- Feasibility of loading other warheads (PBD-dimers, deruxtecan) has been demonstrated

Precise loading of MMAE at one site per chain

Cymirafen data

Cymirafen is very potent – low nanomolar potency against wide variety of human tumor cell lines

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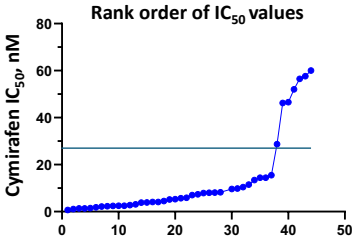

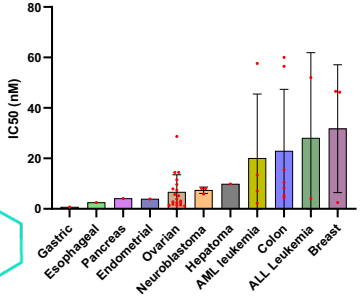

Forty-four cell lines tested to date:

- 31/44 (70 %) have an IC_{50} of ≤10 nM
- 15/44 (34%) have an IC_{50} of <5 nM
- Multiple different cancer types have IC_{50} <10 nM
- Sensitivity is not limited to just one or two types of cancer
- Ovarian and gastric cancers are among the most sensitive in vitro

Nineteen ovarian cancer lines tested to date:

- 15/19 (79%) have an IC50 <10 nM
- 10/19 (53%) have an IC_{50} of ≤10 nM

Cymirafen is more potent against isogenic cancer cells engineered to express higher levels of LGR5

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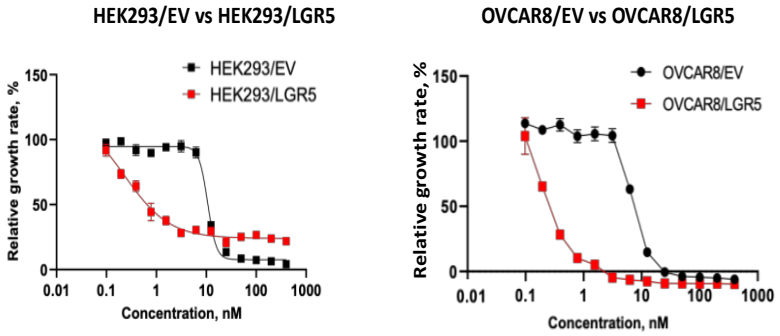
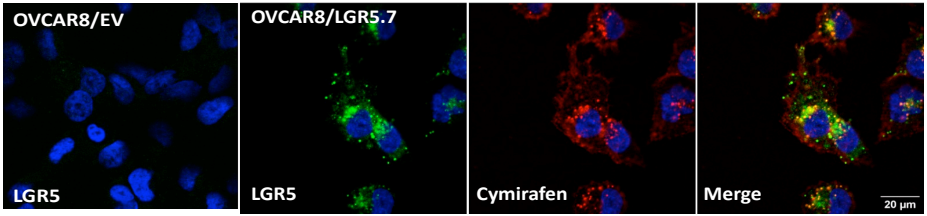


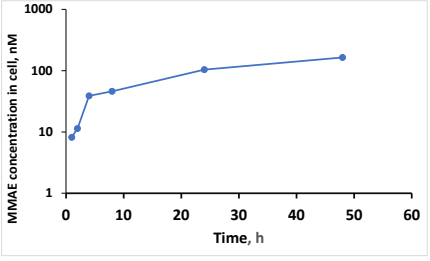
Cytotoxicity to LGR5-low cells is related to endogenous expression of LGR4, LGR6, ZNRF3 and/or RNF43 which are also stem cell markers

Greater potency against LGR5 expressing cells

Cymirafen accumulation is rapid and selective for LGR5 expressing cells



- Cymirafen is found in LGR5-expressing cells within minutes and co-localizes with in lysosomes/endosomes with LGR5



- Free MMAE concentration reached >100 nM which is 100 times higher than the free MMAE IC_{50}

Rapid initial influx, high level free MMAE

Cymirafen kills cancer stem cells

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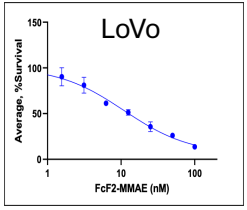
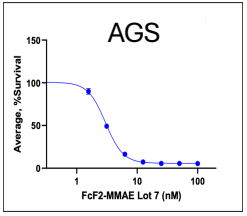
- The ability of a single tumor cell to grow into a large ball (a spheroid) defines it as a cancer stem cell
- Cymirafen kills stem cells at very low nM concentrations in all models tested

Colon



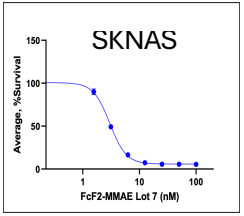
IC_{50} 11.0 nM

Gastric



IC_{50} 3.0 nM

Neuroblastoma



IC_{50} 1.7 nM

Kills stem cells at very low concentrations

Multiple different warheads can be loaded onto cymirafen backbone

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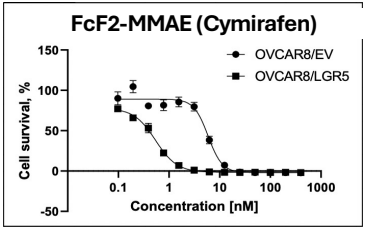

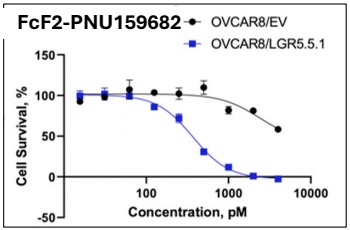

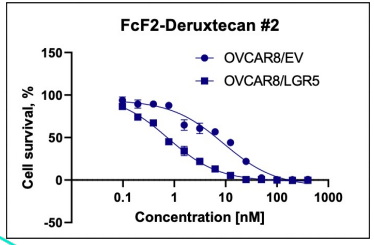

- MMAE, PNU159682 and deruxtecan (Dxd) have all been successfully loaded onto the cymirafen backbone
- **Opportunity to use different warheads for different diseases**

Works with multiple warheads

Cymirafen data:

Efficacy in multiple human xenograft models:

- Documented efficacy in colorectal, gastric, ovarian cancers and neuroblastoma thus far
- IND-enabling studies

Cymirafen efficacy in OVCAR5 high-grade serous ovarian cancer xenograft model

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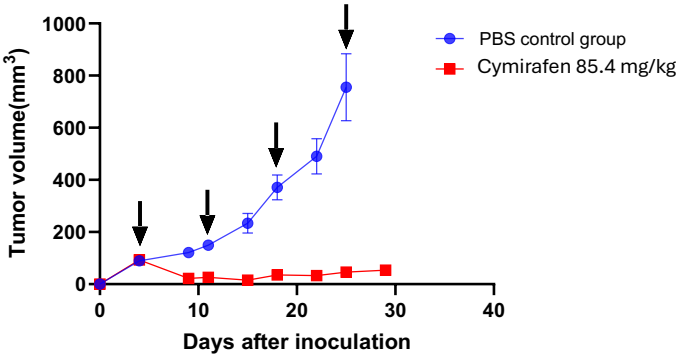
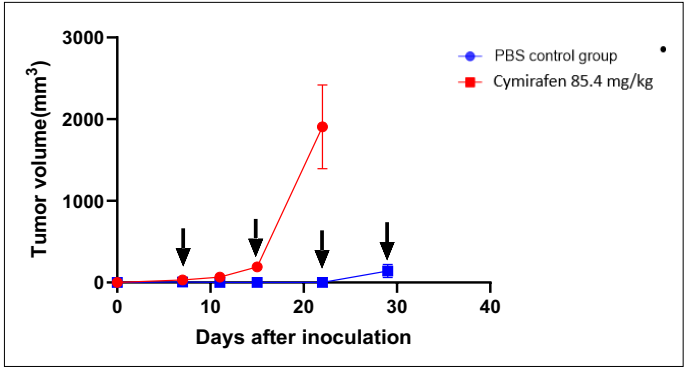


- **Complete inhibition of tumor growth**
- **No significant weight loss** in this model
- **Apparent cure**:
  - Control: 0/16
  - Cymirafen: 16/16

Cymirafen is active in ovarian cancer model

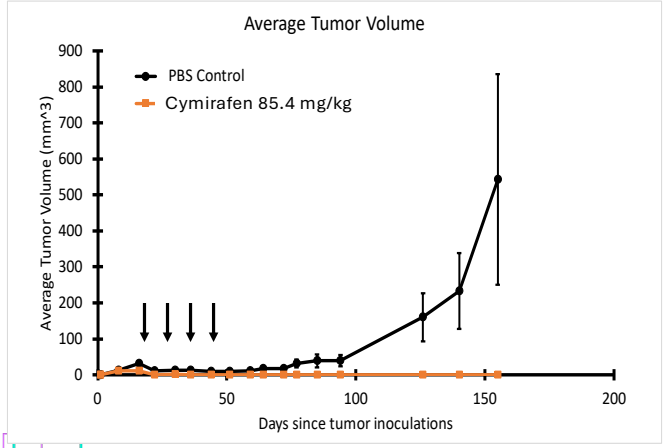
Cymirafen efficacy in A2780 endometrioid ovarian cancer xenograft model



- **Complete inhibition of tumor growth**
- **No significant weight loss** in this model
- **Apparent cure**:
  - Control: 0/16
  - Cymirafen: 9/16

Cymirafen is active in ovarian cancer model

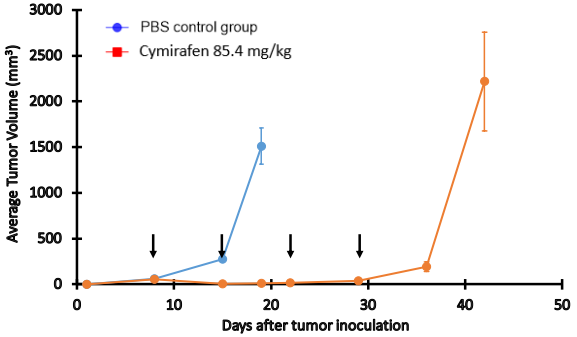
Cymirafen efficacy in AGS gastric cancer xenograft model



- **Marked inhibition of AGS growth**
- Tumor outgrowth: 9/20 vs 0/20, Chi square p value 0.0037
- FcF2-MMAE is active in gastric cancer xenograft model
- **No significant weight loss** in this model

Cymirafen is active in gastric cancer model

Cymirafen efficacy in SKNAS neuroblastoma xenograft model



- Cymirafen produced marked inhibition of SKNAS growth during treatment
- Cymirafen delayed onset of explosive tumor growth by >2-fold
- Cymirafen produced no significant weight loss in this model

Cymirafen is active in neuroblastoma model

Cymirafen data:

Favorable pharmacokinetic profile

Favorable toxicology profile

Wide therapeutic window

Cymirafen single injection dose ranging toxicology in the Sprague-Dawley rat

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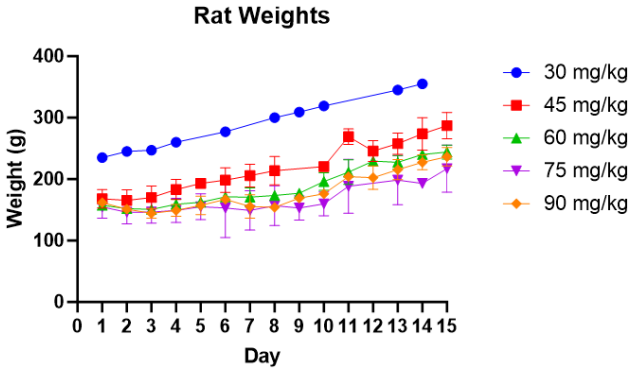

- Dose-related slowing of weight gain; no significant weight loss
- Main adverse event is dose-related neutropenia and thrombocytopenia
- No changes in any serum chemistry measurement
- $HNSDT_{10}$ is ~60 - 75 mg/kg

Cymirafen has greater efficacy against LGR5 rich tumors (isogenic LGR5-low vs LGR5-high isogenic pair)

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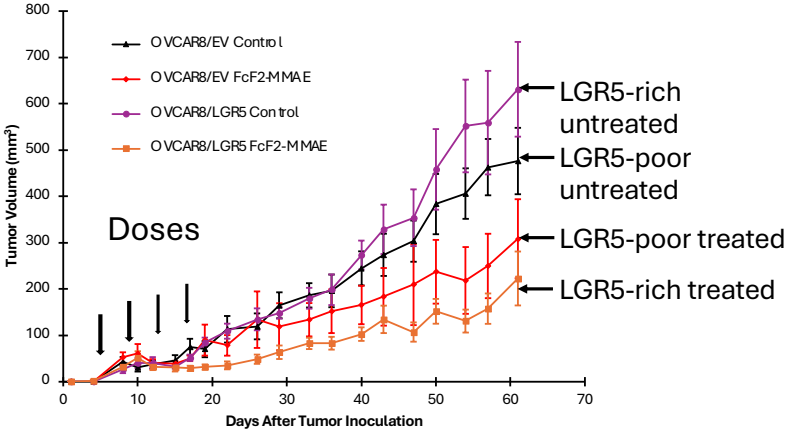

Reduction in growth rate lasts for weeks after end of dosing

Clear differential killing of LGR5-poor vs LGR5-rich tumors

Cymirafen has unique plasma pharmacokinetics in mice & rats

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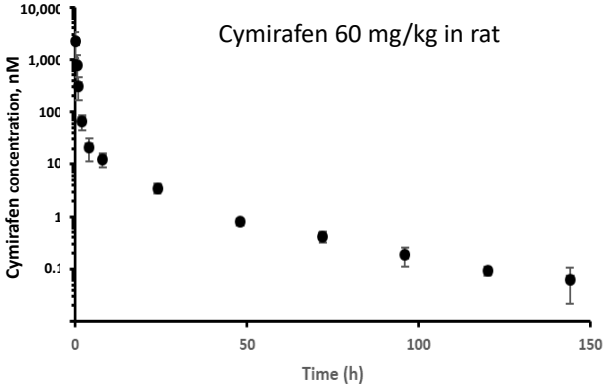
- Cymirafen delivers **extremely high peak concentrations** to tumors for **short** periods of time
- **Reduced risk of neurotoxicity**
- **Plasma stability: rate of release of free MMAE very very low**
  - Only 0.0011 ± 0.0003% free MMAE released in human plasma over 96 h at 37°C
- Terminal half-life in both mice and rats 29 h
- Supports **weekly dosing schedule**



Intense pulse loading of MMAE into tumor without long exposure to plasma free MMAE

All ADCs that use MMAE as a warhead have a problem - neurotoxicity

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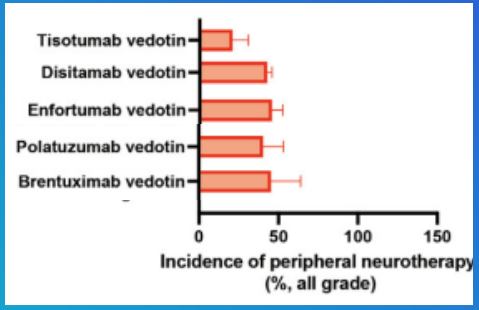
- Doses of ADCs are small (1.2 – 4.5 mg/kg/3 wk) because they are not tolerated
- Doses are too small to penetrate deeply into tumor
- Plasma concentrations are too low to saturate all receptors on those cells the ADC can reach
- Neurotoxicity occurs in up to 50% of patients because they produce very long duration exposure to free MMAE that paralyzes tubulin-mediated neuronal transport

ADC	Brand name	Target	Dose and Schedule	Indication
Brentuximab vedotin	Acetris	CD30	1.8 mg/kg q3w	Multiple lymphomas
Polatuzumab vedotin	Polivy	CD79B	1.8 mg/kg q3w	B-cell lymphoma
Enfortumab vedotin	Paidixiadcev	Nectin	1.25 mg/kg d1, 8,1 5 q mo	Urothelial
Disitamab vedotin	Aidixi	HER2	1.5 mg/kg q1w	Gastric
Tisotumab vedotin	Tivdak	Tissue factor	2.5 mg/kg q3w	Cervical



Delivery of MMAE by cymirafen differs fundamentally from all ADCs that use MMAE as warhead

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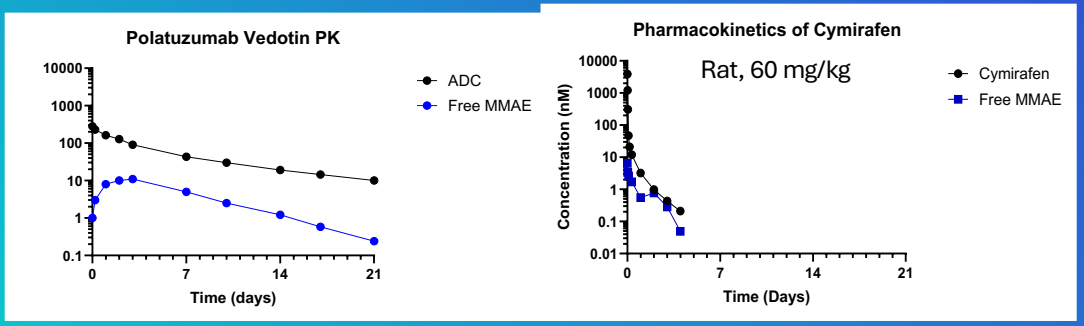

- Cymirafen delivers extremely intense short-duration exposures to MMAE
- Expected benefits:
  - Favors deep penetration and complete saturation of receptors
  - Avoids long exposure to free plasma MMAE that causes neurotoxicity
  - Cymirafen's 2.5-fold higher payload dose loads MMAE into tumors rapidly and to high levels over short periods

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Features of typical MMAE ADC compared to those expected for cymirafen in humans based on allometric scaling

	ADC	Cymirafen*
Dose schedule	1.8 mg/kg q3wk	15 mg/kg q1wk
Cmax, nM	250	1,000
Penetration into tumor	Poor	Much better
Duration of free MMAE >1 nM, days	21	<2
Incidence of neurotoxicity	47%	Much lower or none
***Extrapolated by allometric scaling from rat pharmacokinetics**		

Advantages of cymirafen over LGR5 targeting ADCs

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- **Uses natural ligand** for targeting - **low immunogenicity risk**
- **Cymirafen** can **target** all 3 members of the **LGR family of cancer stem cell receptors** (LGR4, LGR5 and LGR6) and their co-receptors ZNRF3/RNF43 at the same time whereas an ADC can target only a single receptor type
- Can target cells with low expression of one type of the LGR or co-receptor but substantial expression of another
- High affinity bivalent binding of cymirafen to LGR4/LGR5/LGR6 stem cell receptors favors rapid internalization and saturation of tubulin sites
- Ability to bind to ubiquitin ligases independently of LGRs permits **targeting of tumors that uniquely over-express ZNRF3 and RNF43.**
- Attempts to develop LGR5-targeted ADCs have failed due to difficulty of finding high affinity antibodies

Cymirafen Summary and Highlights

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- Specifically attacks **cancer stem cells** - critical target
- Novel concept exploiting **affinity and specificity of RSPO1**
- **High affinity:** Kd **224 pM** for human LGR5
- **High potency,** 0.5 – 10 nanomolar potency against most solid tumors
- **LGR5-dependent killing** in vitro and in vivo
- **Kills stem cells** in spheroid assay (**multiple tumor types)**
- **Limited toxicity** at effective doses on clinically-relevant dose schedule
- **Very different pharmacokinetics from ADCs**
  - Rapid loading of MMAE into tumor; short term exposure to free plasma MMAE
- **High level production** possible in CHO clones
- Limited complexity of **CMC workflow**
- Well paved **regulatory route**

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P: +1.512.394.8512 or +41.76.342.7147
E: info@claytonbiotech.com

CYMIRAFEN - A protein-conjugate cancer therapeutic that selectively targets stem cells in multiple types of cancer

Molecular targets: LGR4, LGR5 and LGR6 receptors uniquely expressed at high levels in cancer stem cells

Cellular target: LGR4, LGR5 or LGR6-positive cancer cells

Indication: gastric, esophageal, colon, endometrial, ovarian, lung, breast, and skin cancers

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